PB82-127564

Inhibition of Benzo(a)Pyrene-Induced Transformation of C3H/10T1/2 Cells by Allylisopropylacetamide and Isopropylvaleramide

Nebraska Univ. Medical Center
Omaha

Prepared for

Health Effects Research Lab.
Research Triangle Park, NC

1981

U.S. Department of Commerce
National Technical Information Service
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TECHNICAL REPORT DATA
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1. REPORT NO. EPA-600/J-80-107	2. JOURNAL ARTICLE
3. REC	PB82-127564
4. TITLE AND SUBTITLE: INHIBITION OF BENZO(a)PYRENE-INDUCED TRANSFORMATION OF C3H/10T1/2 CELLS BY ALLYLISOPROPYLACETAMIDE AND ISOPROPYL-VALERAMIDE	5. REPORT DATE: September 1981
	6. PERFORMING ORGANIZATION CODE: 308054
7. AUTHOR(S): CHARLES KUSZYNSKI, ARPAD SOMOGYI, STEPHEN NESNOW, AND ROBERT LANGENBACH	8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS: Eppley Institute for Research in Cancer, University of Nebraska Medical Center, Omaha, Nebraska 68105	10. PROGRAM ELEMENT NO.: ACSL1A
	11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS: Office of Research and Development (RTP, NC), Health Effects Research Laboratory, US Environmental Protection Agency, Research Triangle Park, North Carolina 27711	13. TYPE OF REPORT AND PERIOD COVERED
	14. SPONSORING AGENCY CODE: EPA-600/11

15. SUPPLEMENTARY NOTES

Published In: Cancer Research 41: 1893-1897, 1981

16. ABSTRACT

Allylisopropylacetamide (AIA) and isopropylvaleramide (IVA) have been demonstrated previously to protect in vivo against the acute toxicity and adrenal necrotic effect of 7,12-dimethylbenz(a)anthracene. In the present study, the influence of these two amides on the in vitro transforming ability of two potent carcinogens, benzo(a)pyrene [B(a)P] and 7, 12-dimethylbenz(a)anthracene, on C3H10T1/2 cells was investigated.. Both AIA and IVA showed a dose-dependent inhibition of B(a)P-incuded transformation of C3H10T1/2 cells when added simultaneously for 24 hr with the carcinogen. While pretreatment, simultaneous treatment, and posttreatment of the cells with AIA or IVA inhibited transformation, the 24-hr post-treatment was somewhat more effective. The protective effect did not appear to result from alterations in B(a)P metabolism inasmuch as aryl hydrocarbon hydroxylase activity and the metabolic products of B(a) P detected by high-pressure liquid chromatography were not changed by AIA or IVA treatment. Furthermore, AIA and IVA did not selectively kill chemically transformed C3H10T1/2 cells, as indicated by the absence of their effect on an established, chemically transormed cell line. AIA and IVA also inhibited 7,12-dimethylbenz(a)-anthracene-induced transformation of C3lH0T1/2 cells. These data suggest that AIA and IVA may be useful protective agents and that they presumably exert their protective effect at some stage between the activation of the carcinogen and the expression of the transformed phenotype.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA 22161

18. DISTRIBUTION STATEMENT: RELEASE TO PUBLIC	19. SECURITY CLASS (This Report): UNCLASSIFIED	21. NO. OF PAGES
	20. SECURITY CLASS (This page): UNCLASSIFIED	22. PRICE

EPA-600/J-80-107
Journal Article

[CANCER RESEARCH 41, 1893-1897, May 1981]
0008-5472/81/0041-0000$02.00

Inhibition of Benzo(*a*)pyrene-induced Transformation of C3H/10T1/2 Cells by Allylisopropylacetamide and Isopropylvaleramide[1]

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ABSTRACT

Allylisopropylacetamide (AIA) and isopropylvaleramide (IVA) have been demonstrated previously to protect *in vivo* against the acute toxicity and adrenal necrotic effect of 7,12-dimethylbenz(*a*)anthracene. In the present study, the influence of these two amides on the *in vitro* transforming ability of two potent carcinogens, benzo(*a*)pyrene [B(a)P] and 7,12-dimethylbenz(*a*)anthracene, on C3H10T½ cells was investigated. Both AIA and IVA showed a dose-dependent inhibition of B(a)P-induced transformation of C3H10T½ cells when added simultaneously for 24 hr with the carcinogen. While pretreatment, simultaneous treatment, and posttreatment of the cells with AIA or IVA inhibited transformation, the 24-hr posttreatment was somewhat more effective. The protective effect did not appear to result from alterations in B(a)P metabolism inasmuch as aryl hydrocarbon hydroxylase activity and the metabolic products of B(a)P detected by high-pressure liquid chromatography were not changed by AIA or IVA treatment. Furthermore, AIA and IVA did not selectively kill chemically transformed C3H10T½ cells, as indicated by the absence of their effect on an established, chemically transformed cell line. AIA and IVA also inhibited 7,12-dimethylbenz(*a*)-anthracene-induced transformation of C3H10T½ cells. These data suggest that AIA and IVA may be useful protective agents and that they presumably exert their protective effect at some stage between the activation of the carcinogen and the expression of the transformed phenotype.

INTRODUCTION

AIA[4] and IVA are aliphatic amides (Chart 1) which have been shown to inhibit hemorrhagic adrenal necrosis and mortality in rats caused by the polycyclic aromatic hydrocarbon DMBA (20). AIA, a synthetic porphyrogenic compound, causes a destruction of hepatic microsomal cytochrome P-450, the substrate-binding site and terminal oxidase of the enzyme system responsible for metabolism of a number of chemicals and drugs, including the polycyclic aromatic hydrocarbons (1, 4, 9, 10). Destruction of cytochrome P-450 is thought to be mediated by an epoxide metabolite of AIA (1, 4, 9), as is the case with several barbiturates, and apparently requires an allyl group in the parent molecule (10). IVA, the structural analog of AIA, contains a propyl group instead of the allyl group and does not affect the levels of cytochrome P-450 in liver microsomes (1, 9). While the effects of these compounds on various biological consequences of carcinogen treatment *in vivo* have been published (20), there are no reports on their possible alteration of carcinogenicity *in vivo* or oncogenic activity *in vitro*. Since these compounds have simple structures and may be the smallest molecules known to protect against hydrocarbon damage, studies were undertaken to determine whether AIA or IVA would affect *in vitro* polycyclic aromatic hydrocarbon-induced oncogenic transformation.

To study the effect of these amides on oncogenic transformation, we have used the C3H/10T½ cell line developed by Reznikoff *et al.* (19). This line has been shown to be transformable by hydrocarbons (18), *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (3), X-rays (21), and antineoplastic agents (5). Furthermore, the effects of agents which modify chemical transformation have been studied with this cell line. Nesnow and Heidelberger (17) reported modification of 3-methylcholanthrene-induced transformation by inducers and inhibitors of microsomal enzymes. Mondal and Heidelberger (14) have demonstrated the effect of the tumor-promoting phorbol esters on UV-induced transformation of these cells, and recently Merriman and Bertram (12) demonstrated a reversible inhibition of 3-methylcholanthrene-induced transformation by retinoids. The inhibition of C3H/10T½ cell transformation by protease inhibitors has also been reported (6).

The present studies describe the results with AIA and IVA on B(a)P- and DMBA-induced transformation of C3H/10T½ cells. The effects of these compounds on B(a)P metabolism, AHH activity, and transformed cell growth are also presented.

MATERIALS AND METHODS

Chemicals. BA and B(a)P, both Gold Label Grade, were obtained from Aldrich Chemical Company (Milwaukee, Wis.). The solvents methanol, hexane, acetone, and chloroform, all spectrograde, were obtained from Burdick and Jackson Laboratories (Muskegon, Mich.). Silation grade DMSO was purchased from Pierce Chemical Company (Rockford, Ill.). The [^{3}H]B(a)P (20 to 40 μCi/mmol) was obtained from Amersham/Searle (Arlington Heights, Ill.). NADPH and DMBA were purchased from Sigma Chemical Company (St. Louis, Mo.). Basal medium (Eagle's) with L-glutamine and phenol red, heat-inactivated fetal bovine serum, and antibiotics (penicillin and strep-

[1] Supported in part by USPHS Contract NO 43-68-959 from the National Cancer Institute, NIH.

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[4] The abbreviations used are: AIA, allylisopropylacetamide (2-allyl-2-isopropylacetamide); IVA, isopropylvaleramide (2-propyl-2-isopropylacetamide); DMBA, 7,12-dimethylbenz(*a*)anthracene; BA, benz(*a*)anthracene; AHH, aryl hydrocarbon hydroxylase; DMSO, dimethyl sulfoxide; T/C, ratio of activity in the treated culture divided by activity in the control culture; PBS, phosphate-buffered saline (0.01 M PBS, pH 7.2, 16 g NaCl, 0.4 g Kcl, 2.3 g Na_2 HPO_4, 0.4 g Kh_2PO_4/2 liters of distilled H_2O).

Received June 26, 1980; accepted February 3, 1981.

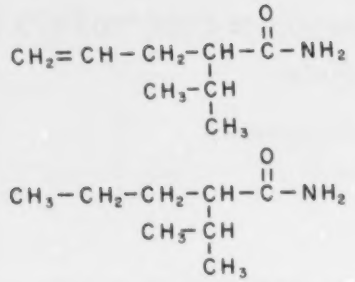

Chart 1. Chemical structures of AIA (*top*) and IVA (bottom).

tomycin) were obtained from Grand Island Biological Company (Grand Island, N. Y.). The Giemsa stain was obtained from Fisher Scientific Co. (Fair Lawn, N. J.).

Cells and Culture Conditions. The mouse embryo cell line C3H/10T½ used in this study was derived from C3H mouse embryos by Reznikoff *et al.* (18, 19), and conditions for cell culture and transformation have been described previously (8, 11). Cells used in all the experiments were between passages 5 and 15 with stock cells maintained in liquid nitrogen. Our cultures of C3H/10T½ cells were found to be free of *Mycoplasma* when tested by Microbiological Associates (Rockville, Md.). The cell cultures were incubated in humidified incubators at 37 ± 0.5° in 5% CO_2 in air. Cells were grown in basal medium (Eagle's) supplemented with 10% heat-inactivated fetal bovine serum containing penicillin (100 units/ml) and streptomycin (50 μg/ml). The supplemented medium is referred to as complete medium.

Transformation Assay. The basic procedure for cell transformation was carried out as described by Reznikoff *et al.* (18). Briefly, 1000 cells/dish (12 or 20 dishes) in a 5 ml complete medium and 200 cells/dish (6 dishes) in 5 ml complete medium were seeded to determine transformation frequency and cloning efficiency, respectively. The cells were treated for 24 hr with the carcinogen beginning 24 hr after cell seeding. The carcinogen was then removed by medium change. The cultures for transformation studies were refed with fresh medium every 6 days for 6 weeks, and fixation with methanol and staining with Giemsa followed. The cloning efficiency dishes were fixed and stained 7 to 10 days after treatment. Three types of morphologically altered colonies of C3H/10T½ cells have been described (18, 19) and, since type III colonies are most tumorigenic, we scored only these foci as transformed. The transformation frequency is expressed as a percentage based on the number of transformed foci, the cloning efficiency, and the number of cells seeded.

In studies in which the effect of modifiers on B(a)P-induced transformation was determined, certain modifications of the treatment regimen were necessary. In studies using simultaneous addition of modifier and carcinogen, both were added 24 hr after initial seeding, and 24 hr later the compounds were removed by medium change. In the experiments with AIA or IVA pretreatment, the modifiers were added by medium change to the cultures 4 hr after cell seeding. At 24 hr after seeding, AIA and IVA were removed, and the carcinogen was added for 24 hr by medium change. The B(a)P was then removed by medium change. For posttreatment of cells, AIA or IVA were added by medium change as the B(a)P was being removed. The AIA or IVA was removed 24 hr later, and the cultures were given medium changes every 6 days, as described above. All control cultures received the medium changes and DMSO at the same concentration (0.5%) used for dissolving the hydrocarbon.

High-Pressure Liquid Chromatographic Analysis of Metabolites. Cells were seeded into 25-sq cm flasks (Falcon Plastics, Cockeysville, Md.) at 50,000 cells/flask with 3 flasks/experimental point, and each point was performed in duplicate. The cells were exposed to the modifier for 24 hr simultaneously with the [^{3}H]B(a)P. The concentration of modifiers used in the metabolite study was 72 μM, a concentration which exerted near-maximal inhibition of transformation when added simultaneously with B(a)P. The [^{3}H]B(a)P concentration was 39 μM, the concentration used for transformation. At the end of the exposure time, the medium was removed, and the cells were trypsinized and lysed by freeze-thawing. The cells and media were combined, extracted with ethyl acetate:acetone (2:1), and centrifuged for 10 min at 1100 × *g*. An aliquot consisting of 80% of the organic layer was removed, evaporated under dry nitrogen, and stored at −80°. High-pressure liquid chromatography was performed on a Dupont Model 848 chromatograph fitted with a 4-mm x 30-cm C_{18}- N-Bondapak column (Waters Associates, Framingham, Mass.). The samples were reconstituted in methanol:acetone (2:1), cold B(a)P metabolite standards were added, and the mixture was chromatographed. The eluting solvent consisted of 66.3% methanol, 30.4% deionized water, and 3.3% ethyl ether run isocratically at ambient temperature and at a flow rate of 1.4 ml/min. The elutant was monitored by UV at 254 nm, and the eluted fractions were collected for liquid scintillation counting. The radiochemical data were quantitated by computer analysis. Typical retention times (min) for each B(a)P metabolite were: 9,10-diol, 3.6; 4,5-diol, 5.5; 11,12-diol, 5.8; 7,8-diol, 6.5; 1,6-quinone, 8.6; 11,12-quinone, 9.1; 3,6-quinone, 9.6; 6,12-quinone, 11.0; 9-phenol, 16.5; 3-phenol, 19.1; B(a)P, 37.5. The overall recovery of radioactivity from the column was greater than 90%.

AHH Assay. To assay the AHH (EC 1.14.1.1) activity in C3H/10T½ cells, the cells were seeded at 1000 cells/ml in 30 ml complete medium into 150-mm tissue culture dishes (Lux Scientific, Newbury Park, Calif.) and grown for 7 days. The following procedure was used to determine the level of AHH activity. The cells from preconfluent cultures were washed twice with PBS, and the cells were then trypsinized, resuspended in PBS, and lysed by freezing and thawing in liquid nitrogen (17). The lysate was assayed for AHH activity by the fluorometric procedure of Nebert and Gelboin (15, 16). The reaction mixture contained 50 μmol Tris buffer, pH 7.5; 0.36 μmol NADPH; 3 μmol $MgCl_2$; and aliquot of the cell lysate as required; 80 μmol B(a)P substrate (in methanol); and enough PBS to bring the total volume of the assay to 1 ml. A lysate equivalent to 2.5 × 10^6 cells was incubated at 37° for 45 min in the assay. This cell number and time of incubation was in the linear region of the respective cell number *versus* activity and incubation time *versus* activity curves. The fluorescence of the extracted metabolites was determined with an Aminco-Bowman spectrophotofluorimeter (American Instrument Company, Silver Spring, Md.) using an excitation wavelength of 394 nm and reading the fluorescence at 520 nm. The resulting fluorescence reading was expressed as a fluorescence equivalent equal to 1 pmol of 3-hydroxybenzo(a)pyrene (15, 16).

The effect of the modifiers on the AHH levels in these cells

was observed by treating the cells with the compounds at intervals from 5 min to 48 hr before cell harvest. The compounds were dissolved in acetone (final concentration, 0.5% acetone) and added in complete medium. Acetone-treated controls were also run. BA, a known inducer of AHH activity, was included as a positive control for induction (17). The results of the assays are expressed as T/C.

Reconstruction Experiment. To determine whether AIA or IVA was active in inhibiting growth of transformed cells, a methylcholanthrene-transformed cell line derived from C3H/10T½ cells (MCA-TCL15) was grown with normal C3H/10T½ cells in the presence of AIA or IVA. Fifty MCA-TCL15 cells and 1000 C3H/10T½ cells were mixed and seeded in 10 ml complete medium per 100-mm Petri dish. Twenty-four hr after plating, the cells were treated with AIA or IVA for 24 hr. The medium containing the chemicals was then removed and replaced with fresh complete medium. At the end of 3 weeks, with medium changes every 6 days, the cultures were fixed and stained, and the colonies of MCA-TCL15 cells on the monolayer of C3H/10T½ cells were counted.

RESULTS

Transformation of C3H/10T½ Cells. The effects on B(a)P-induced transformation of AIA or IVA, when added simultaneously with B(a)P, are shown in Chart 2. Chart 2*A* shows that, at all doses of AIA or IVA tested, there are no significant differences in the plating efficiencies of cultures treated with B(a)P alone (approximately 13%). The plating efficiencies and transformation frequencies for control cultures and AIA- and IVA-treated cultures are given in Table 1.

The data in Chart 2*B* show the inhibition of B(a)P-induced transformation by various concentrations of AIA or IVA. Pretreatment of the cells for 24 hr with 13 μM BA prior to treating with B(a)P (39 μM) yielded a 44% increase over the group treated with B(a)P alone. Treatment of C3H/10T½ cells with BA has been shown previously to increase transformation (17), and this compound was therefore run as a positive control to show that enzyme induction and altered transformation were occurring in each experiment. Both AIA and IVA reduced transformation in a dose-dependent manner at low concentrations but began to level off at higher doses (Chart 2). Because posttreatment with AIA and IVA is most effective in preventing transformation (see below), this leveling off with simultaneous treatment may be due to their removal prior to the time at which they are most effective.

While the above studies demonstrated the ability of AIA and IVA to inhibit transformation, when added simultaneously with B(a)P, it was deemed necessary to determine the effect of pretreatment and posttreatment of these agents on B(a)P-induced transformation. The data in Table 1 demonstrate that neither AIA treatment nor IVA treatment alone for 24 hr increases the transformation frequency above the control level, nor do the compounds alter the plating efficiency of the C3H/10T½ cells. However, pretreatment for 20 hr with AIA or IVA, followed by their removal by medium change prior to B(a)P exposure, resulted in a reduced transformation frequency. IVA (36 μM) pretreatment reduced the transformation frequency by about 62%, while AIA (36 μM) pretreatment reduced the transformation frequency by about 26%. As described in Chart 2, the pretreatment of C3H/10T½ cells with 13 μM BA caused a

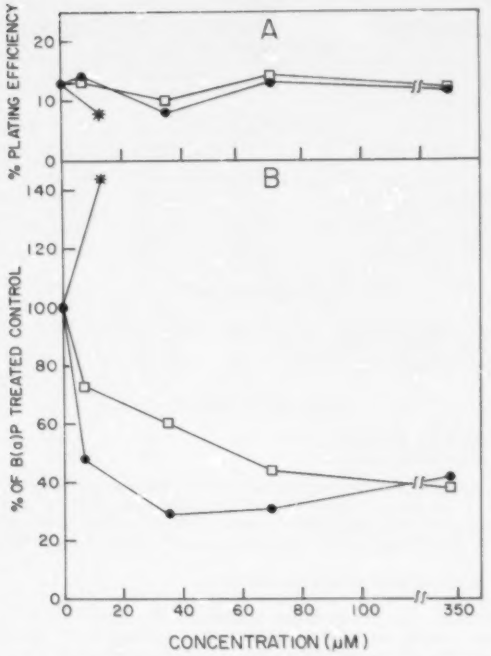

Chart 2. *A*, dose-response effect of AIA and IVA on the plating efficiency of C3H/10T½ cells simultaneously treated with B(a)P. Starting 24 hr postseeding, B(a)P (39 μM) was added to the cell cultures simultaneously with the appropriate concentrations of AIA (□) or IVA (●). BA (*) pretreatment was run as a positive control. The treatment medium was replaced with fresh medium after the 24-hr B(a)P exposure. After 7 to 10 days, the cultures were fixed and stained, and the colonies were counted to determine plating efficiency. *B*, dose-response effect of AIA (□) and IVA (●) on the B(a)P-induced transformation frequency of C3H/10T½ cells. The cells were treated as described above and maintained for 6 weeks, after which the cultures were fixed and stained, and foci were enumerated. Transformation frequencies were determined and calculated as a percentage of the B(a)P-treated control. These results represent a minimum of 2 experiments consisting of 12 dishes/point. The range of the B(a)P-induced transformation for the experiment shown here was less than 10% of the mean. *, BA.

44% increase in the transformation frequency.

Table 1 also shows the effect of a 24-hr posttreatment of AIA and IVA on transformation frequency. In these experiments, the cells were treated for 24 hr with 39 μM B(a)P and, after the carcinogen was removed by medium change, treated for 24 hr with AIA or IVA. The data for AIA (36 μM) posttreatment indicated a 90% reduction in transformation frequency (Table 1), while IVA posttreatment caused a 93% reduction in transformation frequency. Posttreatment studies with AIA and IVA at higher doses (350 μM) also resulted in an approximately 90% reduction in the transformation frequency (data not shown).

Table 2 demonstrates that AIA and IVA can also reduce DMBA-induced transformation. Thus, these agents are effective against 2 hydrocarbons which may have somewhat different pathways of metabolic activation.

Effects on Established Transformed Cells. Since AIA and IVA were capable of reducing the number of transformed foci when added immediately after carcinogen treatment, a reconstruction experiment was carried out to determine their effect on an established transformed cell line. This transformed line was the MCA-TCL15 line originally isolated from C3H/10T½ cells after treatment with a carcinogenic polycyclic aromatic hydrocarbon. The experiment was carried out by mixing 50

Table 1

Effect of pretreatment or posttreatment with AIA or IVA on B(a)P-induced cellular transformation

Compound	PE[a] (%)	No. of colonies: No. of dishes	TF (%)	% of control[b]
	28	2/116	<0.03	<1
B(a)P (39 μM)	13	367/85	3.34	100
BA (13 μM)	27	2/80	<0.03	<1
AIA (36 μM)	24	0/36	<0.03	<1
IVA (36 μM)	24	0/30	<0.03	<1
20-hr pretreatment[c]				
BA (13 μM) + B(a)P (39 μM)	8	132/34	4.82	144
AIA (36 μM) + B(a)P (39 μM)	7	33/18	2.47	74
IVA (36 μM) + B(a)P (39 μM)	9	12/12	1.28	38
24-hr posttreatment				
AIA (36 μM) + B(a)P (39 μM)	11	3/8	0.34	10
IVA (36 μM) + B(a)P (39 μM)	14	4/12	0.24	7

[a] PE, percentage of the cells seeded which survived after treatment, no. of colonies/no. of dishes, number of type III colonies found over the number of dishes examined; TF, transformation frequency defined as the number of transformed colonies divided by the number of cells seeded which survived treatment, times 100%.

[b] TF relative to B(a)P cultures treated alone.

[c] Pretreatment studies were performed by adding AIA, IVA, or BA 4 hr after seeding the C3H10T½ cells. Twenty hr after seeding, the AIA-, IVA-, or BA-containing medium was removed, and medium containing B(a)P (39 μM) was added for 24 hr. The posttreatment cultures received B(a)P for 24 hr beginning 24 hr after seeding. Then the amides were added with a medium change for an additional 24 hr after B(a)P treatment.

Table 2

Effect of AIA and IVA on DMBA-induced transformation

Compound[a]	PE[b] (%)	No. of colonies: No. of dishes	TF (%)	% of control[c]
Control	28	0/20	0	
DMBA (2 μM)	3	18/15	0.4	100
DMBA (2 μM) + AIA (7 μM)	4	7/19	0.09	22.5
DMBA (2 μM) + IVA (7 μM)	3	4/19	0.07	17.5

[a] Cell cultures were treated 24 hr after seeding with a simultaneous addition of DMBA and the amides. Twenty-four hr later, the treatment medium was removed and replaced with fresh medium.

[b] See Table 1, Footnote *a*.

[c] TF relative to DMBA-treated cultures.

MCA-TCL15 cells per 1000 C3H/10T½ cells and seeding them in a 100-mm dish. Twenty-four hr after seeding, the AIA or IVA was added for 24 hr, and the cultures then maintained with medium change every 6 days for 3 weeks. Table 3 shows that neither AIA nor IVA at concentrations which inhibit transformation is capable of inhibiting the growth of the established transformed cell line cultured with normal C3H/10T½ cells under conditions similar to those used for the transformation assay. Studies also showed that these concentrations of AIA and IVA did not affect the population-doubling time of normal or transformed cells when growth curves for pretreated cells, simultaneously treated cells, or continuously treated cultures were compared at 24-hr intervals for 4 days to control cell cultures (data not shown).

AHH Activity and B(a)P Metabolism. Since the compounds did not selectively kill established transformed cells, their effect on cellular transformation is presumably exerted at an earlier

Table 3

Effect of AIA and IVA on cloning efficiency of MCA-TCL15 cells seeded with C3H/10T½ cells

Treatment[a]	Av. no. of colonies/dish[b]	% of PE[c]
Control	20	41
AIA (36 μM)	19	38
IVA (36 μM)	21	42

[a] Treatments were administered 24 hr after the plating of 1000 C3H/10T½ cells with 50 MCA-TCL15 cells. The length of treatment was 24 hr.

[b] Average of the MCA-TCL15 that formed colonies in the dishes (6 dishes/point).

[c] Percentage of the number of MCA-TCL15 cells that formed colonies relative to the number of cells plated.

stage in the transformation sequence. Although posttreatment with these agents did inhibit transformation, it could not be ruled out that some phase of B(a)P metabolism was being inhibited. Modification of the enzymes which metabolize hydrocarbons can either increase or decrease transformation of C3H/10T½ cells (17). Preliminary studies indicated that BA treatment at 13 μM for 24 hr induced AHH activity in C3H/10T½ cells, with the activity declining after 48 hr of continuous treatment. Furthermore, this enhanced activity brought on by 24 hr treatment with BA was transient, and the AHH activity fell to control levels within 12 hr after BA was removed.[5] When dose-effect studies (up to 72 μM) of AIA or IVA on AHH activity in C3H/10T½ cells were done, neither compound had an effect when plotted as a ratio of T/C. BA, a known inducer of AHH activity (17), increased the T/C ratio by more than 6-fold (data not shown).

Since the AHH assays measure the production of certain hydroxylated products of B(a)P, such as 3-hydroxybenzo(*a*)pyrene, another approach to examine the effect of AIA and IVA on hydrocarbon metabolism was to compare quantitatively and qualitatively B(a)P metabolites (9,10-, 4,5-, and 7,8-diols; 1,6, 3,6-, and 6,12-quinones; 3- and 9-phenols) by high-pressure liquid chromatography. When C3H10T½ cells were treated simultaneously with AIA or IVA (72 μM) and [^{3}H]B(a)P (39 μM) for 24 hr, high-pressure liquid chromatography analysis of the organic soluble metabolites indicated no effect of these agents on B(a)P metabolic profiles (data not shown). Furthermore, the extent of B(a)P metabolism to water-soluble products (7) was not altered by these agents. These data, in agreement with earlier studies (20), indicate that AIA and IVA do not manifest their protective effect by altering the metabolism of the hydrocarbons.

DISCUSSION

The data indicate that AIA and IVA can reduce B(a)P- and DMBA-induced transformation of C3H/10T½ cells. It was observed that pretreatment, simultaneous treatment, and immediate posttreatment of the cells with AIA and IVA were all effective in reducing B(a)P-induced transformation, with posttreatment appearing to be most effective. Studies into possible alterations of B(a)P metabolism by AIA and IVA suggested that this was not the mechanism by which they reduced transformation. Somogyi *et al.* (20) also concluded that IVA did not

[5] C. Kuszynski, A. Somogyi, and R. Langenbach. Modification of benzo(a)pyrene-induced transformation of C3H/10T½ cells by pregnenolone-16α-carbonitrile and dexamethasone, manuscript in preparation.

alter DMBA metabolism in the rat adrenal gland or DMBA metabolism by rat liver microsomes. Selective cytotoxicity to transformed cells also seems unlikely inasmuch as neither AIA nor IVA reduced the colony-forming ability of an established transformed 10T½ cell line (Table 3). At present, the mechanism by which these agents manifest their protective effect is unknown.

There is a need to identify new agents which inhibit the carcinogenesis process (22). AIA and IVA, which are probably the smallest molecules known to reverse or prevent the biological effects of chemical carcinogens, may be useful in preventing certain human cancers. While the mechanism by which these agents exert their effect is unknown, it does not appear that AIA or IVA acts in the same manner as do the protease inhibitors (7) or phosphodiesterase inhibitors (2) because only a very short exposure to these compounds is required to inhibit transformation. It is possible that these compounds act by modifying receptors or compete for site in the cells where hydrocarbons interact to initiate the carcinogenic process. The literature (4, 9, 10) supports this possibility since metabolic conversion of AIA to a reactive epoxide intermediate may be responsible for the destruction of the cytochrome P-450. This epoxide could then be responsible for preventing the hydrocarbon epoxide, which is thought to be the ultimate carcinogen form (13), from reacting with the critical cellular constituents which lead to transformation.

It is interesting that the *in vivo* protective effects by AIA and IVA have been observed only for hydrocarbons, for when dimethylnitrosamine, another carcinogen requiring microsomal activation, was tried no protection against *in vivo* cytotoxicity could be obtained (20). Since dimethylnitrosamine is a methylating agent, this lack of inhibition further supports the blocking theory to prevent the interaction of the larger molecule with the cell constituents. Another mechanism by which these amides may protect against hydrocarbon-induced transformation is through alteration of the cellular DNA repair processes. These amides could either induce cellular repair enzymes or cause the repair process to be less error prone. Studies into this possible mechanism are needed.

ACKNOWLEDGMENTS

The authors are grateful to Sharon Leavitt, Betty Hyatt, Linda Montgomery, and Clair Cudak for their technical assistance; to Mardelle Susman for her editorial assistance; and to JoAn Schulte and her staff for typing the manuscript.

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